Persistence of Diflubenzuron on Soybean Leaves[1]

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Abstract Diflubenzuron [Dimilin™, N-[{(4-chlorophenyl)amino}carbonyl]-2-6-difluorobenzamide] is an insect growth regulator that is highly effective and provides residual control (up to 54 days) against velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, on soybean, *Glycine max* (L.) Merrill, in Louisiana. We conducted a study to quantify the field persistence of diflubenzuron residues on soybean leaves when applied at 0.035 kg/ha, a rate used in preventive programs for velvetbean caterpillar. Day of application (day 0) concentration of the insecticide on the leaves was 206 ± 41 ng/cm^2. By 14 days after treatment insecticide leaf coverage had decreased to about 20 ng/cm^2, where it remained for the remainder of the study. This persistent low level residue on the soybean leaves is consistent with the extended diflubenzuron efficacy toward velvetbean caterpillar that has been documented in Louisiana.

Key Words Diflubenzuron, dimilin, soybean, insecticide persistence

Diflubenzuron (Dimilin™), an insect growth regulator, is effective against various insect pests of soybean [*Glycine max* (L.) Merrill], cotton (*Gossypium hirsutum* L.), citrus, vegetables, ornamentals, and forests (both coniferous and deciduous trees) (Crop Protection Handbook, 2003). In Louisiana, some soybean producers routinely apply diflubenzuron at the R2 to R3 growth stage (Fehr et al. 1971) before velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, become established (Willrich et al. 2002). At this stage, soybean are blooming and are attractive to velvetbean caterpillar moths. Velvetbean caterpillar annually migrate from Central and South America and occur in damaging densities in soybean from early to mid-August through September. Diflubenzuron appears most effective in preventive applications on soybean because larval mortality is not immediate and velvetbean caterpillar are rapid defoliators. Boethel (1986) reported efficacy for 54 days after treatment (DAT), and Willrich et al. (2002) observed control for 42 DAT in Louisiana. Foerster (1993; cited in McDonald and Weiland 1995) in Brazil has also reported on the extended efficacy of diflubenzuron against velvetbean caterpillar on soybean. Season-long infestations of velvetbean caterpillar in soybean are negligible when treated with diflubenzuron, at the rate commonly used in preventive programs (0.035 kg ai/ha) (Willrich et al. 2002). Additionally, in a laboratory bioassay, diflubenzuron provided control of beet armyworm,

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Spodoptera exigua Hübner, on cotton after 34 days and 229 mm of rain (Weiland et al. 1996).

Several groups have extracted and determined the persistence of diflubenzuron residues on plant foliage. Table 1 presents results of various field and greenhouse studies with wettable powder formulations. In a greenhouse study Verloop and Ferrell (1977) saw only slight diminution of the insecticide on soybean leaves for 60 DAT; these investigators reported similar results for corn, cabbage, and apple. Bull and Ivie (1978) observed 90% of the initial (day 0) concentration on cotton leaves at 14 DAT; Mansager et al. (1979), however, reported for the insecticide on cotton a half-life of 14 days, followed by a non-diminishing residue after that time. Austin and Hall (1981) reported on persistence of diflubenzuron on apple leaves, finding a half-life of about 14 days. Nigg et al. (1986) observed residues on citrus leaves (Valencia orange) with a half-life >29 days in March-April and 14 days in July-August. Wimmer et al. (1993) observed a biphase persistence on maples, oaks, and yellow poplar (tuliptree) leaves (upper canopy) such that 20% to 80% of the initial coverage disappeared within the first 3 wks after application, followed by a generally persistent residue until leaf fall. Similarly, Prendergast et al. (1995) reported on the upper canopy of oak foliage at $t_{1/2}$ of about 1.3 days until 2 DAT, followed by a persistent residue for the next 118 days. The results of Wimmer et al. (1993) and Prendergast et al. (1995) were complicated by rainstorms within one day of application. Some of these reports on the leaf persistence of diflubenzuron are consistent with the typical but not invariable observation that leaf pesticide residues show an initial rapid disappearance that tapers off to a more or less asymptotic behavior (Willis et al. 1985, Willis and McDowell 1987; Wheatley 1973). This early period after application is the time of volatilization and other physical removal processes preceding a more intimate association with the leaf

Table 1. Persistence of diflubenzuron residues on foliage*

Study	Crop	Residue persistence
Verloop and Ferrell (1977)	soybean	93% remaining, 60 DAT
Verloop and Ferrell (1977)	corn	86% remaining, 60 DAT
Verloop and Ferrell (1977)	cabbage	100% remaining, 60 DAT
Verloop and Ferrell (1977)	apple	86% remaining, 60 DAT
Bull and Ivie (1978)	cotton	90% remaining, 14 DAT
Mansager et al. (1979)	cotton	$t_{1/2}$ = 14 days; residue persistent after 14 DAT
Austin and Carter (1981)	apple	$t_{1/2}$ ca. 14 days
Nigg et al. (1986)	orange	residue persistent for at least 29 DAT, March/April; $t_{1/2}$ = 14 days, July/August
Wimmer et al. (1993)	black oak	$t_{1/2}$ = 1.3 days until 2 DAT; remaining residue persistent, 2-120 DAT
Prendergast et al. (1995)	oak	$t_{1/2}$ ca. 5 days until 8 DAT; remaining residue persistent, 8-141 DAT

* DAT = days after treatment. The WP25 formulation was applied in all studies except Verloop and Ferrell (1977), who applied a wettable powder formulation otherwise unidentified. The investigations were conducted in the field except for Verloop and Ferrell (1977), and Mansager et al. (1979), who carried out their work in greenhouses.

cuticle. Various conditions have been implicated to be important in determining persistence of leaf pesticide residues: rainfall, relative humidity and leaf wetness, solar radiation, heating-degree-days, plant species, and pesticide lipophilic/hydrophilic nature (Willis et al. 1985, Willis and McDowell 1987). Nigg et al. (1986) reported shorter persistence of diflubenzuron during a hot/wet period (July/August) compared to a cool/dry period (March/April). Their results are consistent with higher temperatures (July/August) favoring volatilization and a consequent shorter persistence, but increased humidity, also a condition of their later study period, has been shown to both increase and decrease leaf surface persistence of pesticides (Willis and McDowell 1987). The results of Wimmer et al. (1993) and Prendergast et al. (1995) are consistent with each other. All of these investigations show diflubenzuron to be long persistent on foliage, sometimes with and sometimes without an initial rapid decrease in residues. The non-diminishing persistence of the insecticide on foliage reported by Verloop and Ferrell (1977) and by Bull and Ivie (1978) may be due to application differences, compared to the other reports (Table 1). In both of the earliest citations of the table, labeled insecticide was added to the leaves. Bull and Ivie (1978) describe their careful syringe application to cotton leaves; Verloop and Ferrell (1977) do not describe their procedure for applying labeled diflubenzuron. In the other work listed in Table 1, spray applications of the formulation were made. It is conceivable that syringe application leads to an immediate intimate association of the residue to leaf cuticle; whereas, spray applications take a few days for this close association to occur and allow time for sloughing/volatilization of the residue.

The objective of this study was to determine the field persistence of diflubenzuron residues on soybean foliage. The investigation was conducted in an attempt to relate insecticide leaf coverage to long-lasting control of velvetbean caterpillar by this insecticide as reported by Boethel (1986) and Willrich et al. (2002).

Materials and Methods

Soybeans, "Hartz 6101RR" (Maturity Group VI), were planted at the St. Gabriel Research Station (Iberville Parish) of the LSU AgCenter and managed using agronomic practices and pest control strategies as recommended by the LSU AgCenter. Plots consisted of 4 rows on 91-cm centers and 9.0 m long. Diflubenzuron and non-treated plots were arranged in a randomized block design with 4 replications. On 15 August 2000, diflubenzuron (Dimilin™ 2L, 22.0% [ai wt:wt], Uniroyal, Middlebury, CT) was applied to soybeans (R2 to R3 growth stage) with a hand-held CO_2 sprayer calibrated to deliver 140 L/ha at 207×10^3 Pa (0.035 kg ai/ha) through TeeJet 8002 flat fan nozzles (2 per row).

Soybean leaves (blades with petioles) were collected (20 leaves/plot) from the upper 1/3 of the canopy approximately 3 h after application, on day 1, and then weekly or biweekly for the remainder of the 56-day season. Leaves were stored in locking plastic bags over ice for about 2 hours until they were transported to the laboratory for area measurement and extraction. The single-surface area of the leaves was determined with an LI-3100 area meter (LI-COR Inc., Lincoln, Nebraska). Leaflets were then cut into strips 1-2 cm wide, placed in 1-L jars and covered with 500 mL of ethyl acetate for 3 minutes. The extract was decanted from the leaf pieces, which were then kept covered with an additional 500 mL of ethyl acetate for 3 days. After this time, this second volume of solvent was decanted from the leaf residue. This residue was rinsed three times with additional amounts of solvent and the rinsings were combined with the extended-time extract.

Both extracts were concentrated to 2 mL to afford a pale yellow-green solution (with some suspended solids) from the 3-minute extract and a green-black solution (also containing suspended solids) from the 3-day extraction. The extended extraction removed all of the chlorophyll from the leaf pieces. The concentrated extracts were freed of oils and chlorophyll by elution with 150 mL of hexane:diethyl ether (70:30) through 10% deactivated Florisil (15 g in a 2-cm id glass column). The eluent was concentrated just to dryness, dissolved in 5 mL of benzene, and eluted a second time through another column of Florisil.

For analysis by gas chromatography, diflubenzuron in the extracts was converted to a derivative that lends itself to such analysis. For this purpose, we converted diflubenzuron into *N*-(4-chlorophenyl)trifluoroacetamide by treating the Florisil eluant, after concentration to 5 mL, with trifluoroacetic anhydride in benzene containing trimethylamine. This procedure, described by Smith et al. (1983), afforded a derivatization yield of 60%. The analytical results were divided by 0.6 to correct for the yield in the derivatization step. Recovery of diflubenzuron from soybean leaves spiked with known quantities of the insecticide was >90%. Elution of the insecticide through Florisil was equally efficient.

Extracts, after derivatization, were analyzed with an Agilent Technologies 6890N gas chromatograph with a 15 m long HP-210 capillary column at a temperature of 110°C. The diflubenzuron derivative moved through the column with a retention time of 8.0 min.

The above extraction, derivatization, and analysis procedure was followed with soybean leaves to which a known quantity of diflubenzuron was applied. Dimilin 2L tank mix was added to 20 leaves with a µL-syringe such that 10 µg of diflubenzuron in 50 µL of water was placed on each leaf to give 200 µg of the insecticide on the 20 leaves. Two 20-leaf samples were collected 1 h after treatment, and another two 20-leaf samples were collected 24 h after treatment.

Software for statistical analysis was SAS v. 8, PROCREG (SAS Institute Inc., Cary, NC), and Excel 2000, ANOVA (Microsoft Corporation, Seattle, WA).

Results and Discussion

Approximately 80% recoveries of the applied chemical were obtained when the extraction and analysis procedure was applied to soybean leaves to which a known quantity of diflubenzuron had been added (Table 2). The percentage of material removed by the 3-min dip, compared to that taken up in the 3-d extraction, was 88.4

Table 2. Recoveries (%) of diflubenzuron from spiked leaves

	Time after treatment	
Extraction duration	1 hr	24 hr
3 min	69.8 ± 4.0	71.3 ± 16.8
3 day	10.6 ± 2.3	7.5 ± 2.0
Total	80.4	78.8

± 3.6%, compared to 12.8 ± 4.9%. This ratio indicates that most of the applied insecticide was in an easily accessible location on the leaf surface, and that the remainder was more intimately associated with leaf cuticle. Verloop and Ferrell (1977) report negative evidence for leaf penetration/translocation of ^{3}H-^{14}C-diflubenzuron applied to soybean leaves. Bull and Ivie (1978) and Mansager et al. (1979) have shown with ^{14}C-diflubenzuron that leaf penetration/translocation does not occur on cotton. Results discussed below are consistent with the absence of penetration/translocation of this insecticide on soybean leaves. Except for a trace of rain (producing no leaf drip) on the day of application of diflubenzuron, no rainfall was recorded during the first 7 DAT. A total of 150 mm fell during the 56-day study season.

Diflubenzuron coverage on soybean leaves (Fig. 1) rapidly declined over the first 7 days after application, followed by a persistent residue for the remainder of the season. The figure graphs both 3-min and 3-d extraction results. The regression is the equation for total diflubenzuron leaf content, which is important with respect to velvetbean caterpillar control, because this insecticide exerts its toxicity toward this insect through ingestion. Day 0 total levels averaged 206 ng/cm^2. At 7 DAT these levels of diflubenzuron were at 30.7 ng/cm^2, 14.9% of day 0 coverage. At 56 DAT, the total coverage was 18.0 ng/cm^2, 8.7% of those measured at day 0. The regression is a modified first order disappearance equation; it decays to a constant level of 20 ng/cm^2 after day 20. Consequently, the equation exhibits an increasing half-life for remaining diflubenzuron leaf residues. These half-lives increase from 3 to 6 d in the

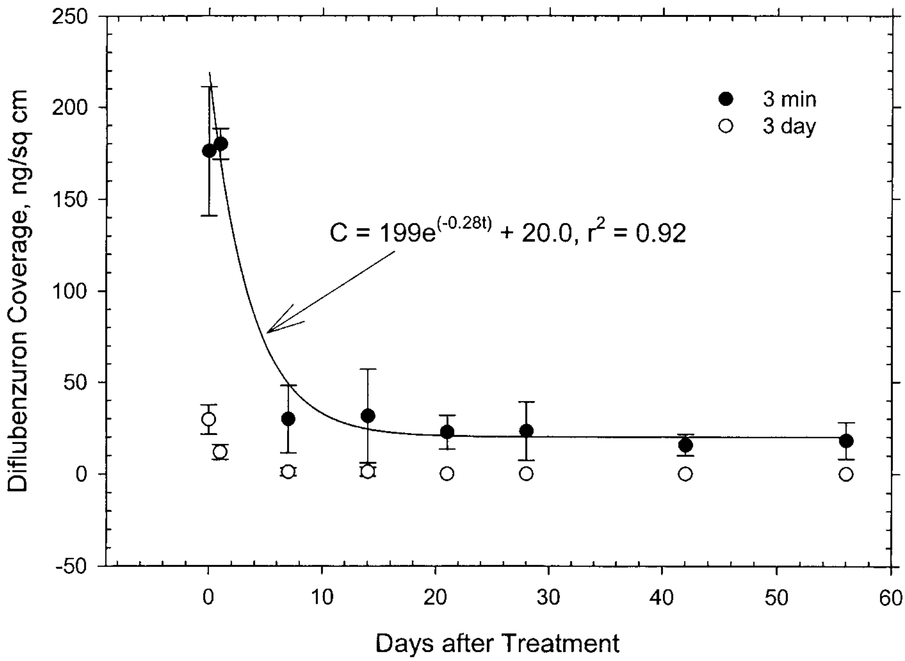

Fig. 1. Diflubenzuron coverage (ng/cm^2) on soybean leaves; 3 minute and 3 day extractions, and regression for total leaf insecticide content.

first 14 d. The equation models an unchanging residue coverage after day 20; the observed difference between 30.7 ng/cm^2 at day 7 and 18.0 ng/cm^2 at day 56 was not significant (Microsoft Excel single factor ANOVA, $P = 0.30$).

From day 21 forward, the 3-d extraction did not remove any diflubenzuron residue. This negative result indicates that at this time leaf coverage was sufficiently small that it was all removed by the 3-min extraction. Leaf penetration by the insecticide should have been revealed by an increasing amount removed by the extended extraction.

Soybean leaf areas (Fig. 2) were not significantly different among sampling dates (Microsoft Excel single factor ANOVA, $P = 0.97$). Mean areas for 20 leaves ranged from 920 cm^2 on 56 DAT to 1090 cm^2 on 14 DAT. Similar leaf areas throughout the study show that leaf expansion did not account for the decrease in coverage that was observed. Soybeans grown in Louisiana are determinate varieties; treatment with diflubenzuron with a long residue persistence after initial decrease is effective because there is minimal new leaf growth. Lauren et al. (1984) reported diminishing diflubenzuron concentrations on alfalfa, *Medicago sativa* L., but concluded that this decrease over their 22-d study season was due to leaf expansion.

Often, as in the data we report here, pesticide residues initially decline rapidly from a leaf surface. This initial rate is due to physical removal of the residue that is in poor contact with the leaf surface. This process is largely due to evaporation, which is influenced by wind, temperature, and relative humidity. A component of this initial disappearance may be caused by sloughing off when leaves rub together, a process especially important with dusts and wettable powders (Ebeling 1963). The initial rate

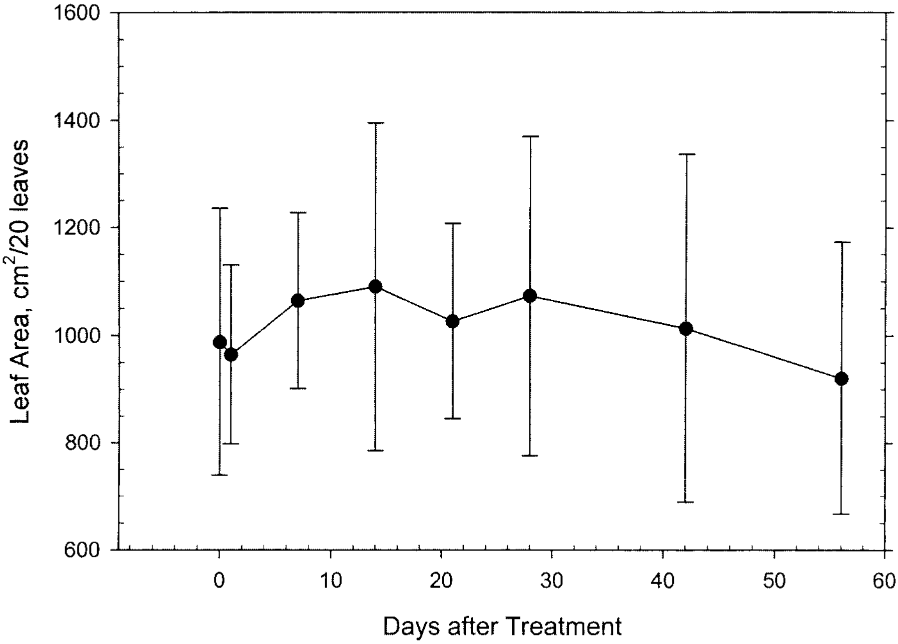

Fig. 2. Areas (cm^2/20 leaves) of soybean leaf samples.

Table 3. Efficacy of diflubenzuron against velvetbean caterpillar*

	Mean number of velvetbean caterpillar larvae/25 sweeps									
	DAT**									
Year, Treatment	3	7	14	21	28	35	42	47	49	54
1986, Dimilin 2F							0.0a	0.3a		0.0a
Dimilin 25WP							0.3a	0.0a		0.0a
Check							14.8b	11.3b		7.3b
1999, Dimilin 2L	2.1a	0.8a	1.0a	0.6a	0.1a	0.3a	0.1a		0.0a	
Check	9.8a	23.9b	46.5b	10.3a	7.0a	16.4b	15.8a		0.0a	
2000, Dimilin 2L	3.8a	0.3a	0.5a	0.0a	0.0a					
Check	9.8a	17.0b	39.3b	33.5b	0.3a					

* Within years, column means (n = 4) followed by the same letter do not significantly differ ($P < 0.05$; Tukey for 1999-2000, Duncan's Multiple Range Test for 1986). The 1986 data, reported by Boethel (1986), were collected at the San Gabriel Research Station after 42 days had elapsed after treatment with Dimilin. The 1999 and 2000 data, collected at the Ben Hur Farms (East Baton Rough Parish) of the LSU Agricultural Center, are graphed in Fig. 4 and 6 of Willrich et al. (2002). Blanks in the table indicate that no sample was collected.

** Days after treatment.

is followed by a slower disappearance such that the overall rate is lower than first order kinetics would predict (Wheatley 1973, Willis and McDowell 1987, Taylor and Glotfelty 1988). In the present work, the third residue half-life (6 d) was twice the first (3 d), followed by a non-diminishing residue from 20 to 56 DAT.

Diflubenzuron exhibits low water solubility (0.08 mg/L) and a high soil sorption coefficient (10000 mL/g) (Hornsby et al. 1996). These properties encourage intimate association with leaf cuticle and provide for a durable leaf residue that is noted for its rainfastness (McDonald and Weiland 1995, Weiland et al. 1996). The initial residue of diflubenzuron on leaves may, however, not be resistant to washoff. Both Wimmer et al. (1993) and Prendergast et al. (1995) reported evidence of washoff of diflubenzuron residues from tree leaves by rainfall. Until intimate association of the residue with leaf cuticle occurs, rainfall washoff/knockoff can occur. In our work, however, the 150 mm of rainfall recorded between 7 and 56 DAT did not apparently influence the residue levels on the soybean leaves (Fig. 1). This low level residue that persists after loss of 90% of the initial coverage is consistent with the extended efficacy against velvetbean caterpillar following applications of diflubenzuron on soybean as reported by Boethel (1986) and Willrich et al. (2002) (Table 3). In these field studies, velvetbean caterpillar infestations in diflubenzuron-treated plots did not reach a level requiring treatment. Compared to non-treated plots, applications of diflubenzuron prevented the establishment of significant infestations of velvetbean caterpillar for ≤54 days in 1986, ≤35 days in 1999, and ≤21 days in 2000.

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